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Weak effects of biochar amendment on soil enzyme activities in mesocosms in bare or *Phleum pratense* soil

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Addition of biochar to soil is studied for the sustainable agriculture. We studied the impact of biochar addition on soil enzyme activities in bare soil and in soil with growing *Phleum pratense* during one season in two experiments, each with different soil types. Enzyme activities were monitored in mesocosms after germination (dry period) and, with fully grown plants, during dry and wet periods. Enzyme activities were high in wet conditions in both soils with growing plants, with or without biochar. Bare soils with or without biochar yielded low activities. In sandy till, alanine and leucine aminopeptidase activities decreased in biochar-treated soil, but not in the medium-fine sand. β-N-acetyl-D-glucosaminidase and phosphomonoesterase activities were enhanced in biochar-treated medium-fine sand. The effects of plant, season and soil type on the enzyme activities were clear and frequently observed, whereas the effects of biochar were only few and weak.

Introduction

Biochars are biological residues degraded under low oxygen conditions, resulting in a porous, low density C-rich material with high surface area and cation exchange capacity (Beesley *et al*. 2011). These properties lead to enhanced sorption of organic and inorganic contaminants, but may also affect nutrient availability. Biochar addition to soil has been recommended as a management approach for improving crop productivity, long-term C sequestration, mitigation of global warming, pathogen management, and for adsorbing signalling molecules and/or as inoculant carriers (Lehmann and Rondon 2006, Lehmann *et al*. 2011). Soil C-mineralisation has been observed to increase in biochar-amended soil, but this was shown to be due to rapid utilisation of a small labile component of biochar (Hamer *et al*. 2004, Cross and Sohi 2011). On the other hand, biochar has also been found to enhance decomposition of added plant residues in soil (Awad *et al*. 2012).

Biochar addition to soil under cultivation of maize during the first year and of grass during the following two years increased above-ground biomass, soil respiration, fungal and bacterial growth rate and turnover in the second year, with a shift towards a bacteria-dominated decomposer community (Jones *et al*. 2012). In paddy soil, biochar addition has been shown to retain more C, with a consequent increase in soil microbial

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activity and crop plant yields (Feng *et al*. 2012). By contrast, on Chernozemic soil or red soils biochar addition did not have a significant effect, or even decreased enzyme activities, whereas straw addition clearly affected all the studied enzyme activities (Wu *et al*. 2013, Zhang *et al*. 2014). These studies suggest that biochar affects microbial enzyme activities in soil mainly indirectly, via increased plant and thus litter productivity.

In boreal environments, biochar was found to increase CH_4 uptake in a short field experiment with a commercial crop (Karhu *et al*. 2011). In a mesocosm experiment with grass, biochar increased biomass and its N content and decreased N_2O emission during the dry period; whereas during the wet period, N uptake decreased and N_2O release increased (Saarnio *et al*. 2013). In bare soil, biochar increased soil moisture, respiration and N_2O emission during the dry period. In these experiments, biochar appeared to affect microbial processes and thus greenhouse gas fluxes by increasing aeration of the soil, increasing or decreasing N uptake of plants and by increasing soil moisture, but the specific effects of biochar on microbial activity in boreal soils are not fully known.

In this paper, we report how moderate biochar addition (10 t ha^{-1}) affected enzyme activities in two sandy soils with or without *Phleum pratense* growths. Sandy till and medium fine sand with different contents of nutrients and organic matter and *P. pratense*, the most common plant species grown on Finnish grass/hay fields, were selected for the mesocosm experiments. Both experiments included dry and wet periods yielding fluctuation in redox conditions, in order to investigate the effects of biochar on enzyme activities under varying aeration conditions. The measured enzyme activities were linked to carbon (α-glucosidase, β-glucosidase, β-xylosidase, cellobiosidase), nitrogen (alanine aminopeptidase, leucine aminopeptidase, β-N-acetyl-D-glucosaminidase), phosphorus (phoshodiesterase, phosphomonoesterase) and sulphur (arylsulphatase) cycling in soils, and to our knowledge the effects of biochar on these enzyme activities have not previously been studied. The biochar used in the experiments had a rather high specific surface area, C:N ratio and pH, which could be expected to increase water holding capacity, pH and nutrient availability in soil, but also to immobilize N. Thus we hypothesized that the addition of fresh biochar would increase microbial activities in soil by providing more available substrates and suitable growth environments for microbes (Steinbeiss *et al*. 2009, Karhu *et al*. 2011, Zimmerman *et al*. 2011) and in dry conditions also indirectly by increasing soil moisture (Karhu *et al*. 2010, Saarnio *et al*. 2013). As the amount of soil organic carbon is one of the main factors controlling enzyme activities in soil (Wallenius *et al*. 2011a, Wallenius *et al*. 2011b), we also expected that growing plants providing exudates and litter, as well as previously accumulated organic matter in soil, would enhance microbial activities. Furthermore, biochar could increase accumulation of new C in soil via increased plant production (e.g. Jeffery *et al*. 2011, Jones *et al*. 2012), thus further enhancing enzyme activities.

Material and methods

Soil characteristics

Two types of soil, sandy till and medium-fine sand, were used in our experiments in 2010 and 2011, respectively. Sandy till, containing higher concentrations of nutrients (except phosphorus) and organic matter, was obtained from an old, over 20 years earlier abandoned field in Mulo, Joensuu, Finland (Table 1). The medium-fine sand containing less organic matter and nutrients (except phosphorus) was taken from an actively cultivated field in Viikki, Helsinki, Finland. In order to remove larger stones and organic litter (roots, straw), sandy till and medium-fine sand were sieved through 7 mm and 3 mm sieves, respectively.

Mesocosms

Two mesocosm experiments, one with 48 mesocosms filled with sandy till and another with 36 mesocosms filled with medium-fine sand (Table 2), were carried out in controlled conditions. Each mesocosm consisted of a 10-cm

diam. and 47-cm long PVC tube closed with a plastic plug at the bottom end. For monitoring the groundwater table, a perforated thin plastic tube (2 cm diam., 50 cm long) was inserted into each mesocosm. The perforated tubes were covered with a polyamide material in order to prevent soil from entering the tube.

In 24 of the 48 mesocosms in the sandy till experiment and in 18 of the 36 mesocosms in the medium-fine sand experiment, biochar was added to the soil at a rate of 7.85 g per mesocosm (i.e. 1000 g m^{-2}, approximately 1% of the soil volume). The remaining mesocosms contained soil only. The biochar was prepared from spruce chips by charring the material at low pyrolysis temperatures for 5–10 minutes so that the final production temperature was 400–450 °C (Preseco Oy, Finland). Most of the biochar was dust, including some larger particles (0.5–4 mm). The final C content was 75% and the N content 0.15%. The specific surface area of biochar was 209.7 m^2 g^{-1} (for more details *see* Saarnio *et al*. 2013).

Half of the mesocosms were sown with *P. pratense*. The *P. pratense* growth was gradually thinned after germination, so that each mesocosm contained 15 shoots. Above-ground plant material was harvested three times during both experiments. All mesocosms were fertilized with YaraMila Nurmen Y 1 N-P-K 20-3-5 fertilizer granules (Yara Suomi Oy, Finland) at the beginning of the experiment and after the first two harvests. Each mesocosm received 0.393 g (50 g m^{-2}) fertilizer granules, the total amount of added N being 10 g m^{-2} per fertilization and 30 g m^{-2} during the whole experiments. This fertilization rate corresponds approximately with Finnish practice in conventional agriculture when

Table 1. Characteristics of the soils.

Characteristic	Sandy till	Medium-fine sand
Total organic carbon (%)	3.74	1.05
Nitrogen (%)	0.25	0.19
Phosphorus (mg l^{-1})	6.8	18
Calcium (mg l^{-1})	1600	1000
Potassium (mg l^{-1})	140	79
Magnesium (mg l^{-1})	100	100
Sulphur (mg l^{-1})	22.2	5.6
Gravel (2–20 mm) (%)	11	0
Coarse sand (0.2–2 mm) (%)	39	19
Fine sand (0.02–0.2 mm) (%)	32	72
Silt (0.002–0.02 mm) (%)	11	2
Clay (< 0.002 mm) (%)	7	7
Conductivity (× 10 mS cm^{-1})	3.1	0.7
pH_{H2O}	5.8	5.9

Table 2. Description of the experiments.

Experiment	Week	Action
Sandy till, 2010	24	Sowing and 1st fertilization (10 g N m^{-2})
	27	Soil sampling for enzyme analyses: Phase 1, *Phleum pratense* germination + dry period
	28	Thinning for 15 seedlings
	33	Soil sampling for enzyme analyses: Phase 2, full grown plant + dry period
	34	1st harvest and 2nd fertilization (10 g N m^{-2})
	39	2nd harvest and raising of the water table
	41	3rd fertilization (10 g N m^{-2})
	45	Soil sampling for enzyme analyses: Phase 3, full grown plant + wet period
Medium fine sand, 2011	23	Sowing and 1st fertilization (10 g N m^{-2})
	24–30	Thinning for 15 seedlings
	27	Soil sampling for enzyme analyses: Phase 1, *Phleum pratense* germination + dry period
	31	1st harvest and 2nd fertilization (10 g N m^{-2})
	34	Soil sampling for enzyme analyses: Phase 2, full grown plant + dry period
	35	2nd harvest, raising of the water table
	37	3rd fertilization (10 g N m^{-2})
	40	Soil sampling for enzyme analyses: Phase 3, full grown plant + wet period

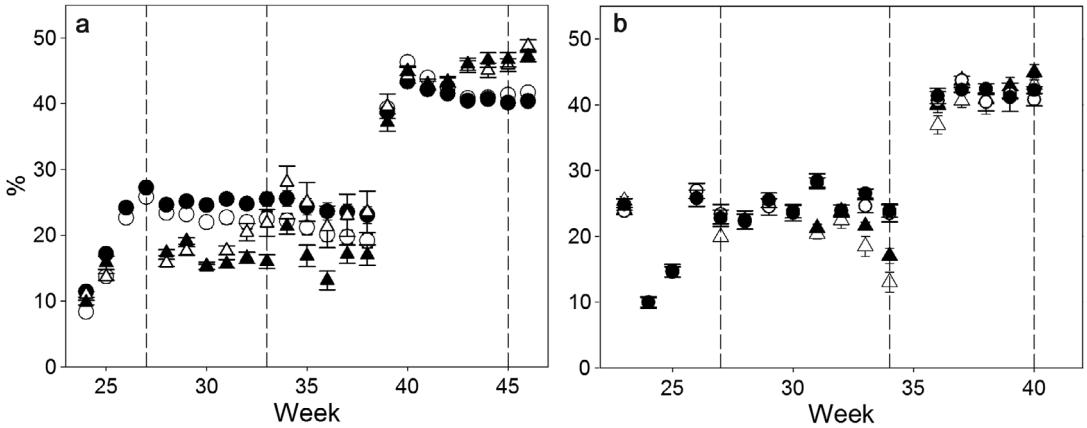

Fig. 1. Mean ± SE soil moisture in the 0–6 cm surface layer in mesocosms during the experiments. (**a**) Sandy till, and (**b**) medium-fine sand. Vertical dashed lines indicate the soil sampling for enzyme activity analyses. ○ = control, ● = biochar, △ = plant, ▲ = biochar + plant.

grass is grown on sandy soils and harvested for silage three times during the growing season.

The mesocosms were irrigated with deionized water. The moisture was measured before the daily (Monday to Friday) irrigation with a Theta Probe type ML2 connected to a Theta Meter type HH1 (Delta-T Devices, Cambridge England). For the first 15 or 12 weeks, the moisture of the mesocosms was maintained at about 20% and after that at 40%–50% (Fig. 1). The groundwater table was monitored via the perforated tubes with a wooden ruler.

Growth conditions

The sandy till experiment was carried out in a controlled environment room (47.3 m^3). The air temperature was set to 20 °C during daytime and 15 °C during night, and humidity to 70% during daytime and 85% during night. The photon flux density was set to change gradually from total darkness to maximum lighting and *vice versa* (for more details *see* Saarnio *et al*. 2013). Mesocosms were transferred to four cylindrical pots (0.23 m^3). Each pot contained 12 mesocosms, three for each of the four treatments. The mesocosms were placed in the pots so that different treatments were equally distributed among different positions. Under the mesocosms there was a ca. 30 cm layer of sand. The sand temperature could be controlled by circulating glycol brine inside a stainless steel coil within the sand layer. Four insulating lids made of plastic foam with 12 holes for the mesocosms, were installed on the top of the pots to maintain the soil temperature at about 15 °C.

The medium-fine sand experiment was carried out in a greenhouse with less accurate controls for air temperature, humidity and photon flux density. The mesocosms were placed in four refrigerators in order to maintain the soil temperature at 15 °C. Each refrigerator contained 9 mesocosms, two to three for each of the four treatments, so that different treatments were equally distributed among different places in every refrigerator.

Soil sampling for enzyme activity measurements

Soil samples from the mesocosms were taken three times during each experiment. Sampling dates represented three growth phases (Table 2). For every sampling time 16 mesocosms in the sandy till experiment and 12 mesocosms in the medium fine sand experiment, four (sandy till) or three (medium fine sand) for every treatment, were taken so that each mesocosm was sampled only once. The samples were taken with a split plastic tube (2 cm diam.) which was set against the wall of the mesocosm and pushed to a depth of 15 cm. The tube was then pulled back up and

the soil layer from 1 to 16 cm was collected. Altogether, one to six subsamples were taken depending on the yield. In the sandy till experiment, the last two samplings from the *P. pratense* mesocosms were taken by cutting the roots with scissors and pulling the sample out. These samples were taken from the 6 cm upper surface layer. In the medium fine sand experiment, the growth of *P. pratense* was weaker and all samples were taken with the split plastic tube. The holes in the soil of mesocosms were filled with the sieved soil. The soil samples were sieved (4 mm) and pieces of roots were removed. Two 4 g samples of soil were frozen (–20 °C) until enzyme activity analyses. The remainder of the collected soil material was used for soil moisture (in 105 °C) and loss on ignition (in 550 °C) analyses (SFS 3008 1990).

Enzyme activity measurements

Enzyme activities were measured from 4 g samples stored in small plastic bags at –20 °C for 80 to 199 days (Wallenius *et al*. 2010) using ZymProfiler® test kits (Vepsäläinen *et al*. 2001, Vepsäläinen *et al*. 2004). We measured the activities of arylsulphatase (Ary), α-glucosidase (α-Glu), β-glucosidase (β-Glu), β-xylosidase (β-Xyl), cellobiosidase (Cell), β-N-acetyl-D-glucosaminidase (NAGase), phoshodiesterase (PDE), phosphomonoesterase (PME), and alanine aminopeptidase (AlaAP) and leucine aminopeptidase (LeuAP). Homogenized samples were suspended in Modified Universal Buffer (MUB), pH 6.5 and 1:100 dilutions were pipetted into multiwells containing pre-dried fluorogenic artificial substrates and incubated with shaking for 3 h at 30 °C. The fluorescence was measured with a Victor^2 multilabel analyzer (Perkin-Elmer) from four replicate wells on the multiwells. For the standardization, the fluorescence curves were assayed with several concentrations of methyl umbellipherone and amino methyl coumarine in three replicates for each sample and dilution. Fluorescence-based enzyme activity measurements have been recommended in biochar studies, even though some measurement uncertainty exists (Bailey *et al*. 2011, Lehmann *et al*. 2011).

Statistical analyses

The effects of the treatments on each enzyme activity were analysed using analysis of variance (ANOVA). ANOVA and Dunnett's test were run separately for the two experiments using Statistix 9 (Analytical Software, Thallassee, USA, www.statistix.com). Only β-xylosidase results needed log-transformation to achieve normal distribution (verified by Shapiro-Wilk's test). The effects of the treatments on the overall enzyme activity pattern were evaluated with cluster analysis. Cluster analysis using Gower's coefficient (Gower 1971) and Ward's method (average linkage method) was applied for enzyme activity data calculated per dry weight using ZymProfiler® programs.

Results and discussion

Analysis of variance for the sandy till experiment indicated several significant changes in enzyme activities among treatments and during the experiment (Table 3). The interaction between treatment and the phase of the experiment was typically significant. The measurements under artificial conditions reflect microbial biomass and accumulation of active enzymes in the soil. The alanine and leucine AP activities were low during Phases 2 and 3 in biochar-amended soil and alanine AP was also low in the control soil during Phase 3, but the activities were high in the soil with plant growth, with or without biochar, during Phase 3. β-Glucosidase, β-xylosidase, cellobiosidase, NAGase and PME activities were elevated in the soil under cultivation during Phase 3, and cellobiosidase and PME in the biochar-amended soil were also elevated. PDE was elevated in the control soil and in the biochar-amended soil during Phase 3. Growth of algae and production of algal PDE on the soil surface in these mesocosms may explain this phenomenon.

No interactions between treatment and phase were found in the medium-fine sand experiment (Table 3). Activities of β-glucosidase, cellobiosidase, PDE, PME and α-glucosidase were elevated during Phase 3, and that of PME already during Phase 2. Plant growth enhanced β-glucosidase and β-xylosidase activities.

Biochar increased NAGase and PME activities during Phase 3, and PME already during Phase 2. β-Xylosidase activity was enhanced in the biochar-amended vegetated soil.

The enzyme activities were generally lower in the medium-fine sand than those in sandy till with higher loss on ignition (Table 4), which is in agreement with the reported increase in enzyme activities with an increase in soil organic carbon content (Wallenius *et al*. 2011a, Wallenius *et al*. 2011b). The addition of biochar was so small that it was not observed as an increase in loss

Table 3. Significant effects of treatment (T) and growth phase (D) on enzyme activities calculated per soil dry weight. ANOVA was used to test for significance of treatments and Dunnett's test was used to identify specific sources of variation (significant at $p < 0.05$). The control soil from the first sampling was used as the control for testing interactions. Phase 1 = Seed germination + dry period; Phase 2 = full grown plant + dry period; Phase 3 = full grown plant + wet period. Significant effect of biochar (BioC) is identified with boldface.

Experiment	Activity	ANOVA			Dunnett's test		
		Source of variation	df	*p*	Multiple comparisons with control	Mean	Difference
Sandy till	AlaAP	D × T	6	< 0.001	Phase 2/**BioC**	1.148	–0.589
					Phase 3/Control	1.140	–0.597
					Phase 3/**BioC**	1.062	–0.675
					Phase 3/**BioC** + Plant	2.300	0.563
					Phase 3/Plant	2.472	0.735
	β-Glu	D × T	6	0.030	Phase 1/Plant	2.230	0.505
					Phase 3/Plant	2.257	0.533
	lg β-Xyl	D × T	6	0.004	Phase 3/Plant	–0.369	0.156
	Cell	D × T	6	0.006	Phase 3/**BioC** + Plant	0.330	0.092
					Phase 3/Plant	0.332	0.095
	NAGase	D × T	6	0.002	Phase 3/Plant	0.630	0.203
	LeuAP	D × T	6	< 0.001	Phase 2/**BioC**	0.705	–0.378
					Phase 3/**BioC**	0.809	–0.274
					Phase 3/**BioC** + Plant	1.555	0.472
					Phase 3/Plant	1.652	0.569
	PDE	D × T	6	< 0.001	Phase 3/Control	1.852	1.469
					Phase 3/**BioC**	1.408	1.025
	PME	D × T	6	0.002	Phase 3/**BioC** + Plant	4.972	1.642
					Phase 3/Plant	4.815	1.485
	α-Glu	T	3	0.041	Not different[1)]		
	Ary	T	3	0.013	Not different[1)]		
Medium fine sand	β-Glu	D	2	0.025	Phase 3[2)]	1.210	0.206
		T	3	0.036	Plant[1)]	1.205	0.244
	lg β-Xyl	D	2	0.027	Not different[2)]		
		T	3	0.036	**BioC** + Plant[1)]	–0.640	0.076
					Plant[1)]	–0.636	0.080
	Cell	D	2	0.001	Phase 3[2)]	0.167	0.052
	NAGase	D	2	0.002	Phase 3/**BioC**[2)]	0.371	0.150
	PDE	D	2	0.004	Phase 3[2)]	0.299	0.074
	PME	D	2	< 0.001	Phase 2/**BioC**	3.276	1.349
					Phase 3/**BioC**	3.309	1.381
					Phase 3/**BioC** + Plant	3.637	1.710
					Phase 3/Plant	3.658	1.730
					Phase 2[2)]	2.851	0.541
					Phase 3[2)]	3.418	1.639
	α-Glu	D	2	0.019	Phase 3[2)]	0.115	0.023
	Ary	D	2	0.001	Phase 2[2)]	0.071	–0.017

[1)] Control soil as the control.
[2)] The first sampling as the control.

on ignition. The mean activities in the medium-fine sand were for arylsulphatase 94%, PME 77%, β-xylosidase 68%, α-glucosidase 63%, β-glucosidase and alanine AP 60%, leucine AP 56%, cellobiosidase 53% and PDE 42% of the activities in sandy till. Correspondingly, CO_2 and N_2O effluxes were higher from sandy till than from medium-fine sand and from vegetated mesocosms (Saarnio *et al*. 2013; S. Saarnio unpubl. data). The fivefold growth of *Phleum pratense* and possibly thus better C allocation in the soil in sandy till than in medium-fine sand may have contributed to the difference in the enzyme activities between the soils.

Cluster analysis was run separately for each experiment (Figs. 2 and 3). There was some variation between replicate mesocosms, and all the replicates did not always belong to the same cluster. However, in sandy till (Fig. 2) there was a tendency towards elevated enzyme activities in mesocosms with plant growth — with and without biochar — during wet Phase 3 (the lowest main cluster), whereas low activities (with the exception of PDE) occurred simultaneously in the control and the biochar-only treated soils (the main cluster in the middle). During germination and the dry period (Phase 1), enzyme activities tended to be elevated in the *P. pratense* soil. PDE activity was elevated in the control and in the biochar-only treated soil during the wet period (Phase 3). Phase 1 and Phase 2 samples tended to cluster together irrespectively of the treatment. Similarly, in medium-fine sand (Fig. 3), enzyme activities tended to be high in the soil with plant growth — with and without biochar — late in the season and low early in the season.

Plant growth enhanced enzyme activities during wet conditions late in the season (Phase 3) in both experiments, which can be attributed to a rhizosphere effect. Biochar appeared not to increase enzyme activities any further in the soil with plant growth, and PDE in sandy till, and NAGase and PME in medium-fine sand were the only activities to be enhanced in the biochar-treated soil without plants, mainly during Phase 3. However, this plant- or biochar-induced increase in P- and N-mineralising enzyme activities during the wet phase was not observed in the rate of respiration in either soil (Saarnio *et al*. 2013; S. Saarnio unpubl. data).

Overall, biochar at the application level used had only a few and weak effects on enzyme activities. Simultaneously, the effects of plant growth and season were mostly significant indicating the sensitivity of the enzyme-activity analysis. Aminopeptidase activities showed a temporary slight decrease in sandy till supplemented with biochar. This decreased activity of enzymes related to the decomposition of N compounds was supported by the slightly decreased N_2O efflux from the biochar-amended bare soil during the wet period (Saarnio *et al*. 2013). It is also in agreement with our expectation that the addition of biochar may immobilize N. In medium-fine sand, biochar increased NAGase activity late in the season and PME during Phases 2 and 3. This is in agreement with observations that biochar addition alone does not affect enzyme activities in the soil (Awad *et al*. 2012). However, if plant residues were also added, biochar significantly increased enzyme activities in both studied soil types. Wu *et al*. (2013) used a similar dose of biochar to that used in our study in barren Chernozemic soil and after a 100-day incubation found no effect on dehydrogenase or β-glucosidase and a decrease in urease activity. This is in agreement

Table 4. Mean ± SE loss of ignition (%) in soil samples. For sandy till $n = 4$ and for medium fine sand $n = 3$.

Experiment	Phase	Control	Biochar	Plant	Biochar + Plant
Sandy till	1	8.1 ± 0.2	8.1 ± 0.2	8.2 ± 0.1	8.1 ± 0.1
	2	7.8 ± 0.2	8.1 ± 0.2	7.8 ± 0.3	8.0 ± 0.3
	3	7.9 ± 0.3	8.2 ± 0.3	7.8 ± 0.1	7.9 ± 0.2
	mean	7.9	8.1	7.9	8.0
Medium fine sand	1	5.7 ± 0	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0
	2	5.8 ± 0	6.0 ± 0.1	5.7 ± 0.1	5.9 ± 0
	3	5.7 ± 0	5.9 ± 0.1	5.7 ± 0	5.9 ± 0
	mean	5.7	5.8	5.7	5.8

Sample	Ary	LeuAP	AlaAP	PME	PDE	α-Glu	Cell	β-Xyl	β-Glu	NAGase
Control P1	0.08	1.03	1.71	3.11	0.33	0.15	0.22	0.28	1.51	0.33
Control P1	0.08	1.01	1.62	3.00	0.32	0.15	0.18	0.27	1.57	0.29
BioCharPlant P1	0.09	1.05	1.57	3.17	0.34	0.14	0.20	0.21	1.60	0.36
BioChar P1	0.06	1.04	1.62	3.26	0.41	0.13	0.23	0.28	1.58	0.37
Control P2	0.09	0.85	1.43	3.34	0.48	0.16	0.24	0.32	1.76	0.34
Control P2	0.10	0.88	1.44	2.90	0.32	0.15	0.23	0.33	1.77	0.34
BioCharP2	0.09	0.72	1.23	3.38	0.44	0.14	0.21	0.23	1.74	0.38
BioCharPlantP1	0.08	1.00	1.47	3.19	0.38	0.16	0.24	0.29	1.67	0.44
BioCharPlant P1	0.09	1.04	1.62	3.52	0.49	0.16	0.23	0.26	1.85	0.39
Control P2	0.08	1.12	1.86	3.43	0.37	0.16	0.23	0.30	1.90	0.34
Plant P2	0.08	1.22	1.87	3.85	0.36	0.15	0.22	0.26	1.84	0.34
BioCharPlantP1	0.09	1.30	1.95	3.26	0.36	0.16	0.23	0.29	1.63	0.38
BioCharPlant P2	0.07	1.13	1.97	4.01	0.42	0.15	0.24	0.34	1.84	0.44
BioCharPlant P2	0.08	1.21	1.93	4.15	0.35	0.16	0.27	0.31	1.90	0.42
BioChar P1	0.08	1.21	1.91	3.32	0.40	0.13	0.32	0.36	1.88	0.37
Control P1	0.10	1.11	1.71	3.69	0.43	0.17	0.31	0.33	1.89	0.53
BioChar P1	0.10	1.03	1.54	3.54	0.45	0.17	0.29	0.30	1.98	0.48
Control P1	0.09	1.18	1.90	3.53	0.44	0.16	0.24	0.32	1.92	0.56
BioCharPlant P2	0.09	1.13	1.79	4.00	0.48	0.18	0.27	0.32	2.16	0.37
Plant P2	0.09	1.02	1.70	3.95	0.42	0.18	0.26	0.30	1.90	0.39
PlantP2	0.09	1.14	1.82	4.48	0.45	0.17	0.25	0.26	1.82	0.46
Plant P1	0.09	1.01	1.59	4.42	0.38	0.17	0.28	0.39	2.23	0.46
BioChar P2	0.09	0.84	1.29	4.11	0.63	0.20	0.30	0.35	2.11	0.62
Control P3	0.10	0.90	1.30	3.12	1.69	0.19	0.23	0.35	1.87	0.37
Control P3	0.09	0.80	1.21	2.94	2.36	0.16	0.20	0.30	1.53	0.32
BioChar P3	0.08	0.83	1.14	3.43	2.27	0.16	0.26	0.27	1.47	0.32
Control P3	0.08	0.73	0.86	2.59	2.09	0.16	0.19	0.26	1.35	0.30
BioChar P3	0.07	0.83	0.93	3.12	2.30	0.12	0.20	0.24	1.52	0.29
BioChar P2	0.05	0.60	0.95	2.24	0.30	0.12	0.13	0.16	1.15	0.24
BioCharP2	0.07	0.66	1.12	1.88	0.23	0.12	0.14	0.20	1.14	0.24
BioChar P3	0.07	0.71	1.06	2.59	0.47	0.12	0.21	0.30	1.50	0.32
BioChar P3	0.08	0.87	1.12	2.48	0.58	0.14	0.20	0.27	1.59	0.32
Control P2	0.07	0.78	1.29	2.87	0.31	0.11	0.17	0.23	1.42	0.33
Control P3	0.06	0.87	1.19	3.12	1.27	0.09	0.16	0.24	1.33	0.27
BioCharPlantP3	0.10	1.77	2.38	4.89	0.38	0.15	0.29	0.40	1.82	0.50
Plant P3	0.10	1.55	2.33	4.35	0.45	0.16	0.29	0.40	1.99	0.55
PlantP1	0.10	1.61	2.31	4.20	0.44	0.15	0.31	0.39	2.45	0.49
PlantP1	0.10	1.37	2.21	3.96	0.45	0.16	0.30	0.33	1.97	0.47
BioCharPlant P3	0.08	1.39	2.23	4.69	0.40	0.15	0.28	0.31	2.03	0.51
BioCharPlantP3	0.07	1.43	2.19	4.02	0.41	0.15	0.35	0.29	1.84	0.61
BioCharPlantP2	0.10	1.08	1.66	4.88	0.47	0.20	0.34	0.39	2.11	0.48
PlantP2	0.11	1.24	1.87	4.86	0.63	0.21	0.34	0.39	2.18	0.49
BioChar P1	0.10	1.21	1.88	3.89	0.53	0.18	0.30	0.46	2.09	0.54
Plant P1	0.11	1.34	1.94	4.10	0.43	0.17	0.33	0.43	2.27	0.48
Plant P3	0.11	1.56	2.18	5.40	0.46	0.18	0.37	0.47	2.29	0.57
PlantP3	0.09	1.88	2.93	4.82	0.57	0.18	0.34	0.44	2.57	0.75
PlantP3	0.10	1.62	2.46	4.68	0.58	0.17	0.34	0.41	2.18	0.65
BioCharPlant P3	0.12	1.63	2.41	6.29	0.64	0.20	0.40	0.47	2.63	0.70

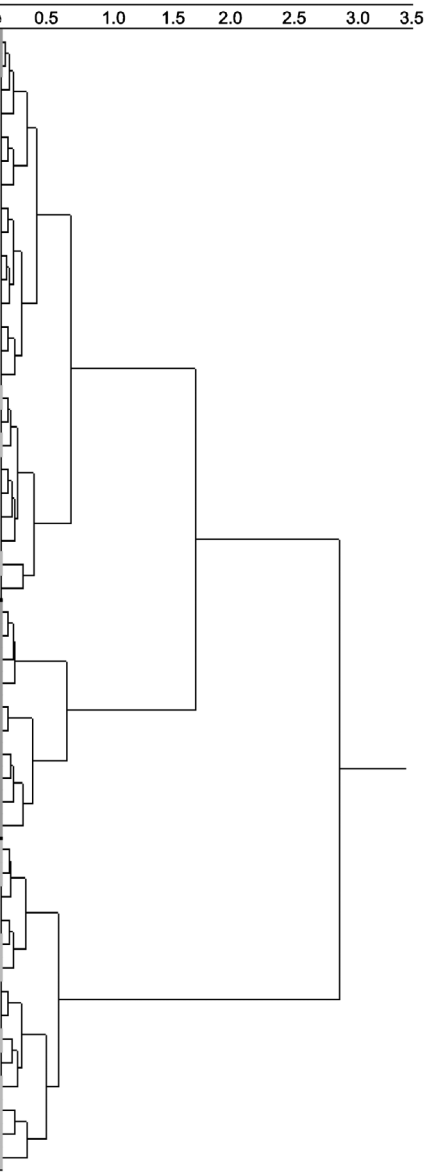

Fig. 2. Cluster analysis results of enzyme activities in the sandy till experiment. Standardised data calculated per dry weight using Gower's coefficient and Ward's method (similarity index above the dendrogram; the shorter the horizontal lines connecting the samples in the dendrogram, the more similar are the samples or the clusters). Means of replicate measurements of enzyme activities are in μmol MUF (for AlaAP and LeuAP AMC)/g dry soil/3h are given. Upper quartiles are on the dark-grey and lower quartiles on light-grey backgrounds. P = phase, *see* Table 2 for sampling phases.

with our results of only a slight effect of biochar, as no fresh litter was added and the biochar-induced increase in plant growth (and thus in litter production) was observed only on sandy till and only temporarily in Phase 1 (Saarnio *et al.* 2013; S. Saarnio unpubl. data).

The same enzyme activity potential measurements as used in our study were used in some earlier studies. Contrary to the present study, the activities of β-glucosidase, PME and NAGase were found to decreased during summer, whereas the leucine AP activity increased towards the

Sample	Ary	LeuAP	AlaAP	PME	PDE	α-Glu	Cell	β-Xyl	β-Glu	NAGase
Control P1	0.09	0.62	0.90	1.57	0.18	0.09	0.11	0.19	0.85	0.22
BioCharPlant P1	0.08	0.62	1.00	2.33	0.19	0.09	0.10	0.18	0.86	0.23
Control P1	0.09	0.67	0.96	1.49	0.17	0.07	0.10	0.17	0.85	0.18
BioChar P1	0.09	0.64	0.95	1.71	0.18	0.11	0.10	0.23	0.96	0.19
BioChar P1	0.10	0.50	0.72	1.89	0.21	0.06	0.10	0.19	1.03	0.21
Plant P1	0.09	0.51	0.82	1.86	0.22	0.09	0.12	0.20	0.94	0.25
BioCharPlant P1	0.07	0.47	0.70	2.64	0.21	0.09	0.08	0.18	0.85	0.22
Control P2	0.06	0.32	0.45	2.06	0.17	0.06	0.10	0.16	0.70	0.18
Plant P2	0.07	1.04	1.32	2.56	0.22	0.09	0.10	0.19	1.10	0.24
Plant P2	0.07	0.96	1.52	2.69	0.23	0.07	0.15	0.18	1.03	0.24
BioChar P2	0.07	0.95	1.33	2.34	0.21	0.07	0.09	0.17	0.80	0.21
BioChar P2	0.07	0.41	0.66	2.91	0.28	0.09	0.15	0.20	1.22	0.36
BioChar P3	0.09	0.37	0.58	2.87	0.30	0.09	0.13	0.18	1.05	0.35
BioCharPlant P2	0.07	0.45	0.82	2.91	0.26	0.09	0.17	0.25	1.07	0.27
BioChar P3	0.08	0.49	0.89	2.87	0.26	0.08	0.19	0.22	1.04	0.34
BioChar P1	0.10	0.51	1.07	2.39	0.22	0.09	0.13	0.23	1.05	0.33
BioCharPlant P1	0.11	0.64	1.04	2.25	0.22	0.10	0.14	0.23	1.19	0.27
Control P3	0.08	0.70	1.09	3.63	0.24	0.09	0.18	0.20	1.01	0.30
Control P3	0.08	0.66	1.02	2.51	0.21	0.11	0.15	0.21	1.12	0.29
Control P2	0.07	0.64	0.96	2.90	0.25	0.09	0.13	0.19	0.87	0.27
BioChar P2	0.07	0.68	1.14	2.78	0.27	0.10	0.13	0.21	1.01	0.26
Control P1	0.08	0.60	0.98	2.72	0.26	0.11	0.13	0.19	0.97	0.25
Control P2	0.06	0.62	1.01	2.69	0.35	0.09	0.14	0.20	0.99	0.26
Plant P3	0.09	0.73	1.18	4.28	0.31	0.14	0.18	0.29	1.52	0.40
BioCharPlant P3	0.09	0.81	1.41	3.92	0.35	0.14	0.19	0.28	1.31	0.39
BioChar P3	0.11	0.88	1.43	4.19	0.40	0.16	0.23	0.31	1.47	0.42
Control P3	0.09	0.59	0.95	3.06	0.31	0.13	0.19	0.24	1.29	0.32
BioCharPlant P3	0.07	0.57	1.07	3.86	0.30	0.13	0.17	0.23	1.18	0.33
Plant P1	0.09	0.63	0.96	3.86	0.29	0.11	0.18	0.25	1.34	0.35
BioCharPlant P2	0.08	0.61	1.08	3.57	0.32	0.12	0.16	0.25	1.27	0.40
Plant P1	0.08	0.65	1.08	3.00	0.36	0.11	0.12	0.31	1.18	0.31
BioCharPlant P3	0.08	0.74	1.19	3.14	0.30	0.10	0.17	0.24	1.14	0.32
Plant P2	0.09	0.59	1.02	3.44	0.29	0.11	0.06	0.23	1.35	0.32
Plant P3	0.09	0.63	1.18	3.51	0.34	0.11	0.13	0.23	1.33	0.32
BioCharPlant P2	0.07	0.65	1.15	3.35	0.30	0.09	0.11	0.22	1.48	0.33
Plant P3	0.08	0.53	1.00	3.18	0.28	0.12	0.10	0.23	1.05	0.29

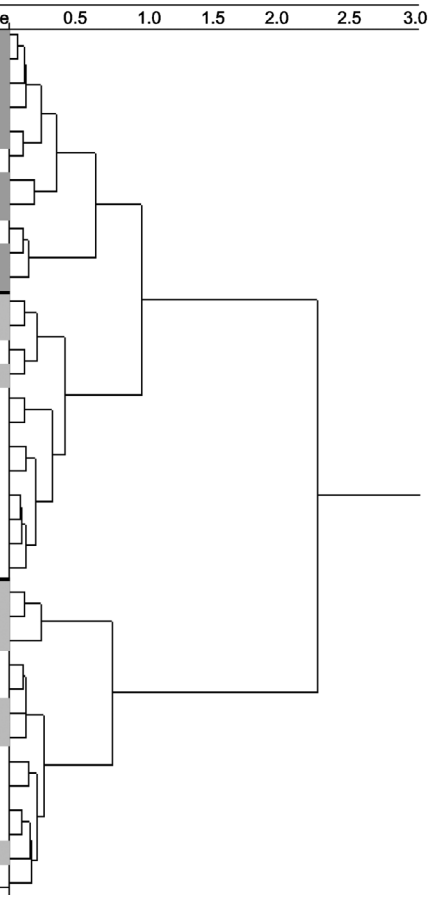

Fig. 3. Cluster analysis results of enzyme activities in the medium fine sand experiment. Standardised data calculated per dry weight using Gower's coefficient and Ward's method (similarity index above the dendrogram; the shorter the horizontal lines connecting the samples in the dendrogram, the more similar are the samples or the clusters). Means of replicate measurements of enzyme activities are in μmol MUF (for AlaAP and LeuAP AMC)/g dry soil/3h are given. Upper quartiles are on the dark-grey and lower quartiles on light-grey backgrounds. P = phase, *see* Table 2 for sampling phases.

end of summer and the arylsulphatase activity was at its highest in the middle of summer in *P. pratense* and *Trifolium pratense* fields in fertile fine sand (Niemi *et al*. 2005). The differences in the enzyme activity dynamics between these studies may be due to the difference in variation in the soil moisture and soil chemical properties. In the silt-clay soil, cellobiosidase, β-xylosidase, β-glucosidase and arylsulphatase had high activities in the peat-amended plots, probably due to substrate availability in peat (Vepsäläinen *et al*. 2004), but biochar did not cause a similar stimulation in our study. The enzyme activities were generally higher, arylsulphatase and PME activities consistently so, in the organic cropping than in the conventional cropping system in the silt soil (Niemi *et al*. 2008). Other enzyme activities were higher during either the first or the second year or seasonally. The peat amendment increased PME, PDE, leucine AP, NAGase, cellobiosidase and α-glucosidase activities but decreased arylsulphatase and initially alanine AP activities, whereas β-glucosidase and β-xylosidase activities were increased only during the 3rd year of the experiment. Biochar in our short-duration experiments had only a slight effect on PME.

A very limited effect of biochar on the mineralisation rate of low molecular weight dissolved organic N compounds in two agro-ecosystems was found by Dempster *et al*. (2012), and this

is in agreement with our slightly decreased aminopeptidase activity results. The biochar treatment had a minimal effect on microbial parameters and global greenhouse-gas fluxes during the first 14 months after biochar incorporation in a Mediterranean wheat crop experiment (Castaldi *et al*. 2011). In this field experiment with high spatial variability, observed differences were rarely significant. Spatial heterogeneity could be controlled better in our mesocosm study, but statistically significant effects on microbial activity were rare. On the other hand, the biochar treatment did not interfere with plant-induced stimulation.

Although the effects on soil enzyme activities were only minor in both experiments, biochar significantly affected soil moisture, yield and N content of *P. pratense*, ecosystem respiration and N_2O emission in the same mesocosms (Saarnio *et al*. 2013; S. Saarnio unpubl. data), which was considered to affect or reflect microbial activities in soil. However, these changes were too slight and temporary to result in detectable changes in soil enzyme activities during one season. By contrast, the effects of plant, season and soil type on enzyme activities were clear and frequently observed. This illustrates the sensitivity of the enzyme activity analysis and confirms that biochar had few and weak effects on soil enzyme activities. This may also indicate that this biochar could be used to store C in hayfields, as it does not interfere with plant growth and nutrient cycling in the soil.

The observed weak effect of biochar addition on soil enzyme activities, generally reflecting sensitively soil management practices, necessitates further investigation. Several reports indicate the need for further long-term controlled studies on the use of biochar for soil management. Humification as well as C and N degradation processes of grass-derived pyrogenic organic material were followed for 28 months in soil by Hilscher and Knicker (2011). Specific degradation processes altered the molecular structures of biochar material, affecting the chemical and physical properties of the char residue and making it more available for further microbial attack but also for adsorption processes. Biochar application and reapplication rates affect nutrient dynamics and microbial growth in soil (Quilliam *et al*. 2012). The biomass selected as the source material and the pyrolysis method used both affect the properties of biochar and its effects on soil biota (Lehmann *et al*. 2011, Bruun *et al*. 2012). Soil characteristics and other management processes affect the impact of biochar (Beesley *et al*. 2011). Therefore, further studies are needed to compare different biochar types, to follow the long-term impacts on soil processes of biochar addition and for optimisation of the treatment (addition level and frequency) in different well-characterised soil types.

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